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A revision of Bithyniidae (Mollusca, Gastropoda) from the Inle Lake Basin, Myanmar

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Abstract

Inle Lake, located on the Shan Plateau of Myanmar, is the only ancient lake on the Indochinese Peninsula and a biodiversity hotspot of freshwater fauna with high diversity and endemism, including molluscs. However, recent research on its biodiversity remains limited. Based on morphological, anatomical, and molecular studies, we systematically revise the freshwater snails of the family Bithyniidae from the Inle Lake basin. We provide an updated re-description of four bithyniid species within three genera from the Inle Lake basin, including one new record, *Digoniostoma iravadica* **comb. nov.** The endemic genus *Parabithynia* **syn. nov.** with one endemic species of Inle Lake, *Hydrobioides physcus* **comb. rev.**, is considered a synonym of *Hydrobioides*. *Gabbia nana* **comb. nov.** is assigned to *Gabbia* based on shell morphology and molecular phylogeny. *Hydrobioides dautzenbergi* **stat. rev.** from Thailand is recognised as a valid species rather than a synonym of *Hydrobioides nassa*, which is endemic to Shan State of Myanmar. There are altogether seven bithyniid species recorded from the Inle Lake basin and surrounding area, including four endemic species.

Key Words

Ancient lake, bithyniid snail, phylogeny, radula, Shan State, taxonomy

Introduction

Inle Lake, located on the southern Shan Plateau, is the second largest lake in Myanmar and the only ancient lake on the Indochinese Peninsula, which was formed around 1.5 million years ago; despite its relatively small size (covering about 116 square kilometres) and shallow depth (averaging only two meters), Inle Lake supports remarkable freshwater biodiversity and endemism, similar to other ancient lakes such as Baikal, Tanganyika, and Biwa (Hampton et al. 2018). There are 13 families and 28 genera of fish native to the Inle Lake basin, including 15 endemic species (Kano et al. 2016); 36 species of freshwater molluscs, including 18 endemic species, have been recorded from the Inle Lake basin (Annandale 1918; Annandale and Rao 1925).

Bithyniidae is one of the most common families of freshwater snails in the Inle Lake basin. This diverse and common family of freshwater snails is widely distributed across Africa, Eurasia, and Australia, inhabiting rivers, wetlands, ponds, and lakes. It also includes *Helicostoa*, the only known group of obligate sessile freshwater snails (Zhang et al. 2024a). Annandale (1918) recorded and described altogether six bithyniid species from the Inle Lake basin and surrounding areas: *Hydrobioides turrita* (Blanford, 1869) (subfossils from Heho), *Hydrobioides nassa*

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(Theobald, 1866), *Parabithynia physcus* (Annandale, 1918), *Hydrobioides avarix* Annandale, 1918, *Hydrobioides nana* Annandale, 1918, and *Gabbia alticola* (Annandale, 1918). Of these, four species (*P. physcus*, *H. avarix*, *H. nana*, *G. alticola*) and one genus, *Parabithynia* Pilsbry, 1928, were considered endemic to the Inle Lake basin. Sawada (2022) revisited Annandale's historical malacological collection from Inle Lake, kept in the Kyoto University Museum, including the dry-shell specimens of three bithyniid species (*H. nassa*, *P. physcus*, *G. alticola*). However, since the works of Annandale and Rao (Annandale and Rao 1925; Rao 1928), there have been almost no published modern studies and collected records of freshwater snails from the Inle Lake basin or even Shan State.

In July 2024, we conducted comprehensive fieldwork for freshwater fauna in the Inle Lake basin, collecting a diverse assemblage of freshwater molluscs, including bithyniid snails. The present study aims to provide a systematic revision and re-description of bithyniid snails from the Inle Lake basin based on shell morphology, anatomy, and molecular study, after a nearly century-long gap in research on this biodiversity hotspot of freshwater fauna.

Materials and methods

Materials

Seven type specimens of three species (one for *Hydrobioides nassa*, three for *Hydrobioides physcus*, three for *Hydrobioides avarix*) were examined from the Natural History Museum, London (**NHMUK**). Fieldwork was conducted in the Inle Lake basin from 1 Jul. 2024 to 11 Jul. 2024. The samples of bithyniid snails were collected in five localities (Fig. 1B); the types of habitats included lake, rice field, shallow pond, wetland, and river (details see in Systematic Descriptions). The samples were fixed using 75% ethanol and then preserved in analytical pure ethanol. Additional materials for comparison were collected in Mandalay Region and mountains of Kalaw Township (Shan State) (details see in Systematic descriptions: *Hydrobioides nassa*, *Digoniostoma iravadica*). The newly collected specimens are deposited in the Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming (**KIZ**), and the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw (**SEABRI**).

Examination of morphology

The shell height (H) and width (W) were measured with a calliper to a precision of 0.01 mm. The specimens were photographed in a consistent orientation using focus stacking methods with a Nikon® Z5 camera and a Nikon® Nikkor Z MC 105mm f/2.8 VR lens. S. Zerene Stacker 1.04 software was used to do focus stacking. Radulae were extracted through dissection; radulae were cleaned by boiling in 1% NaOH solution for half an hour and rinsed with distilled water. Radulae and operculum were coated with gold before scanning electron microscopy with a Zeiss® Sigma 300 scanning electronic microscope. The male genital structure of the snail was examined during dissection and photographed under a stereomicroscope. The terms used for describing the operculum are according to Zhang and von Rintelen (2021). The newly collected specimens were identified based on the description of shell, radula, male genitalia, and collecting locality provided by Annandale (1918), Rao (1989), and comparisons to type specimens. Abbreviations of radula: **ct**–central teeth; **lt**–lateral teeth; **imt**–inner marginal teeth; **omt**–outer marginal teeth.

Molecular methods

DNA was extracted from 10–20 mg of foot tissue of each snail using a mollusc-specific CTAB/chloroform 112 extraction protocol (Winnepenninckx et al. 1993). A fragment of the mitochondrial 16S gene was amplified through polymerase chain reaction (PCR) with the following primer pairs: LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′, and COX-B7R, 5′-ACCACCAGCTGGATCAAAAA-3′ (Schultheiß et al. 2011) for COI; 16Sar-L, 5′-CGCCTGTTTATCAAAAACAT-3′, and 16Sbr-H, 5′-CCGGTCTGAACTCAGATCACGT-3′ (Palumbi et al. 2002). PCR amplifications were conducted in volumes of 30 μL under the following cycling conditions: an initial denaturing step at 94 °C for 10 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min (COI) or 52 °C for 30 s (for 16S), and 72 °C for 1 min, with a final extension step of 10 min at 72 °C. The purification and sequencing were conducted by Sangon Biotech.

Sequence and phylogenetic analyses

New sequences of four species have been uploaded to GenBank; 46 sequences of 25 bithyniid species were included in the present study (Suppl. material 1). *Amnicola dalli* (AY622435) of family Amnicolidae was selected as the outgroup based on Zhang et al. (2024b). Sequences were aligned using MUSCLE as implemented in Geneious (https://www.geneious.com). Genetic distances were calculated using MEGA X (Kumar et al. 2018). GTR+G was suggested as the best-fit model of sequence evolution for our dataset by means of the Akaike and Bayesian information criteria in MEGA X. A Bayesian inference (BI) analysis was performed with MrBayes 3.2.6 (Ronquist et al. 2012) as implemented in Geneious with four independent chains for 5,000,000 generations, sample freq = 1,000, burnin = 25%, and it was confirmed that convergence was reached based on the trace plots generated in Geneious Prime 2020. A maximum likelihood (ML) analysis was conducted with RAxML as implemented in Geneious, with support estimated by 1,000 bootstrap replicates (Kozlov et al. 2019).

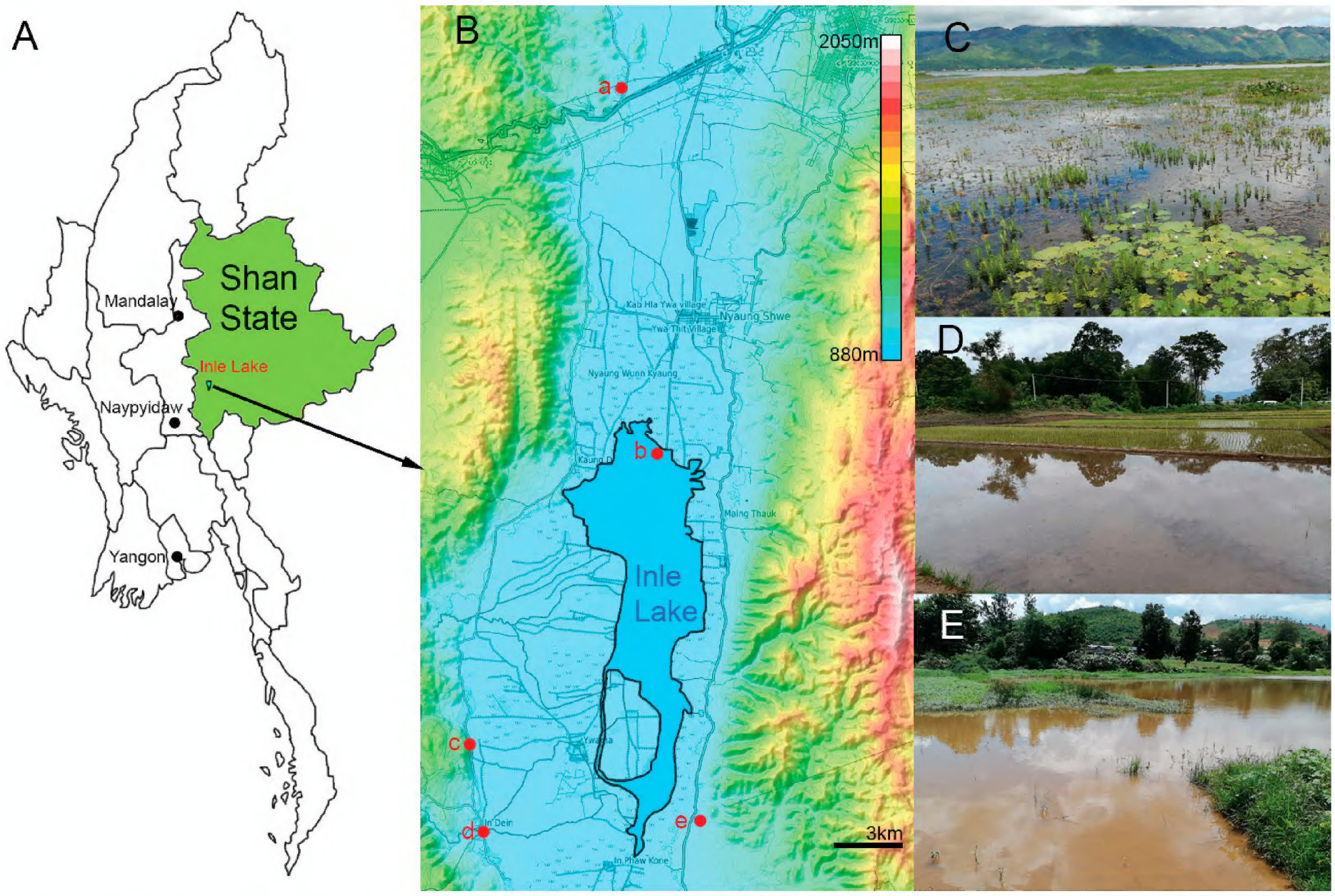

Figure 1. Collecting sites of bithyniid snails in the Inle Lake basin. **A.** A map of Myanmar showing the location of Shan State and Inle Lake; **B.** A red dot (site a to e) showing the collecting sites in the Inle Lake basin, an altitude map modified based on the resources from https://topographic-map.com (license offered by the website builder Guillaume VK); **C.** Habitat at the site b, Inle Lake; **D.** Habitat at the site e, rice field; **E.** Habitat at the site a, shallow pond.

Results

Molecular phylogeny

The molecular tree (Fig. 2) reveals that *Hydrobioides* is not a monophyletic group. *Hydrobioides dautzenbergi* is the sister group of a clade including species of *Hydrobioides*, *Gabbia*, and *Digoniostoma*. *Hydrobioides nassa* is the sister species of *Hydrobioides physcus*. *Gabbia nana* is a distinct species, clustered with *Gabbia pygmaea*. *Digoniostoma* is a highly supported monophyletic group, which is sister to genus *Gabbia*. *Digoniostoma iravadica* is a distinct species clustered with the clade including *Digoniostoma funiculata*, *Digoniostoma siamensis siamensis*, and *Digoniostoma siamensis goniomphalos*. Four subfamilies of Bithyniidae (Parafossarulinae, Bithyninae, Helicostoinae, Mysorellinae) are generally supported by the tree. All the species from the Inle Lake basin belong to the subfamily Mysorellinae.

Systematic descriptions

Class Gastropoda Cuvier, 1795
Order Littorinimorpha Golikov & Starobogatov, 1975
Family Bithyniidae Gray, 1857

Subfamily Mysorellinae Annandale, 1920

***Hydrobioides* Nevill, 1885**

Hydrobioides Nevill, 1885: 42.
Paranerita Annandale, 1920: 4.
Parabithynia Pilsbry, 1928, syn. nov.: 108.
Bithynia (*Parabithynia*) – Popova et al. 1970: 95.

Type species. *Fairbankia turrita* Blanford, 1869 (original designation; Myanmar, Irrawaddy River, "Kyoukpong").

Diagnosis. Shell medium for the family, solid, aperture lip thickened, outer lip outward extended; penial appendix located in the central of penis, penial appendix slightly shorter than distal part of penis.

Remarks. According to Molluscabase (2024a), there are six extant valid species assigned to this genus; five species are described from Myanmar, and one species is described from Laos. The type species of this genus is *Hydrobioides turrita* (Blanford, 1869) with high spiral whorls and an extremely outward extended outer lip (Fig. 3P); its type locality is "Kyoukpong" near the Irrawaddy River in Myanmar (probably referring to Kyouk Pon near Bagan in the Mandalay Region), and it was also collected from Heho near the Inle Lake basin as subfos-

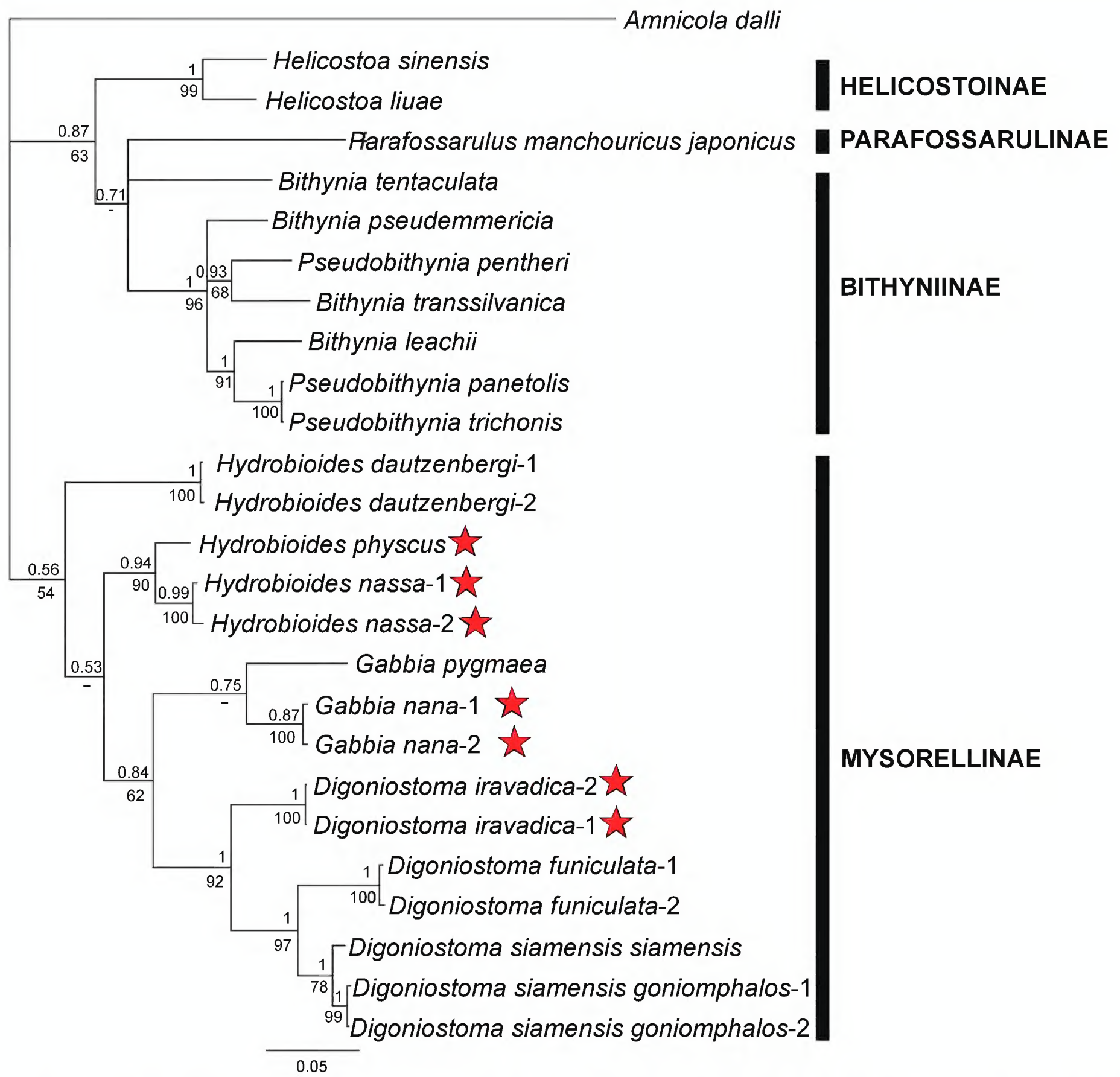

Figure 2. Bayesian inference phylogram based on partial COI and 16S sequences of the selected bithyniid snails, with an emphasis on species from the Inle Lake basin (red star). Numbers above branches are BI posterior probabilities, and numbers underneath branches are ML bootstrap values.

sils (Annandale 1918). However, this species has never been collected again since Annandale (1918). Based on shell characters of *Hydrobioides turrita*, we consider that the outward extended outer lip is the most important morphological diagnosis character for this genus. *Hydrobioides nassa* is always considered widely distributed in north to central Thailand, but we consider that the Thai population should be another species (see in remarks of *Hydrobioides nassa*). We consider that *Parabithynia* Pilsbry, 1928, should be a synonym of *Hydrobioides* (see in remarks of *Hydrobioides physcus*). The molecular study based on 16S and COI does not support *Hydrobioides* as a monophyletic group (Fig. 2). The taxonomy of this genus needs systematic revision.

Hydrobioides nassa (Theobald, 1866)

Bithinia nassa Theobald, 1866: 275 (Myanmar, Shan State).

Hydrobioides nassa – Annandale, 1918: 118–120, pl. 13, figs 1–7, pl. 14, figs 4, 4a.

Hydrobioides nassa distoma Annandale, 1918 †: 120, pl. 13, fig. 1 (Myanmar, Shan States, Heho plain).

Hydrobioides nassa lacustris Annandale, 1918: 119, pl. 13, figs 4, 4a, 5, 5a, 7 (Myanmar, Shan States, Inle Lake).

Hydrobioides nassa rivicola Annandale, 1918: 119–120, pl. 13, figs 6, 6a (Myanmar, Shan States, small streams at Thamakan).

Diagnosis. Shell medium, high spiral whorls, a varix near outer lip, outer lip outward extended.

Material examined. • 1 ***syntype*** (preserved dry), Upper Salwin River, Purchase of W. Theobald, NHMUK.1888.12.4.845; 3 specimens (preserved in ethanol), Shan State, Taunggyi District, Taungpo-etgyi, near Tone Lae, river near spring (Fig. 1B. c), 20°29'48.9"N, 96°50'17.5"E, 2 Jul. 2024, Le-Jia Zhang leg., KIZ.2400054–2400056 • 2 specimens (preserved in ethanol), Shan State, Taunggyi District, Nyaungshwe, Inle Lake, floating islands near Intha (Fig. 1B. b), 20°36'33.4"N, 96°54'53.8"E, 3 Jul. 2024, Le-Jia Zhang leg., KIZ.2400057–2400058 • 2 specimens (preserved in ethanol), Shan State, Taunggyi District, Kalaw, river near Nanthe' Cafe Garden, 20°36'30.6"N, 96°33'55.3"E, 11 Jul. 2024, Le-Jia Zhang leg., KIZ.2400059–2400060.

Description. Shell (Fig. 3) medium for the family (Table 1), thin but solid, conical, bright yellow or olive grey, with five whorls at adulthood; teleoconch relatively high, smooth, without shoulder, a weak to strong varix structure on the body whorl, close to or directly connected to the outer lip; aperture ovate, lip thickened, nearly half of shell in height, outer lip outward extended; umbilicus covered by inner lip completely.

Operculum (Fig. 3E, 4A) calcium, ovate, thin, slightly smaller than aperture, transparent grey; exterior surface smooth, nucleus close to the inner opercular margin; interior surface with wide concentric veins on inner opercular region, nuclear region relatively small, with irregular veins.

Penis (Fig. 5A) with a grey penial appendix located in the central of penis, penial appendix slightly shorter than distal part of penis.

Radula (Fig. 6) taenioglossate (2 + 1 + 1 + 1 + 2); central tooth upper margin with one broad triangle central cusp and two to three small sharp cusps on either side, lateral margin each with two to three small sharp cusps; lateral tooth with one broad tongue-shape central cusp and three to four small sharp cusps; inner marginal tooth with 14 to 15 small cusps; outer marginal tooth with eight to nine small cusps.

Remarks. This species can be easily distinguished from other bithyniid snails based on the varix structure near the outer lip. The location of varix can be relatively far from the outer lip (Fig. 3I–M), viz. "*Hydrobioides nassa lacustris*" (Fig. 3N), mostly found in Inle Lake; the varix also can be directly connected to the outer lip (Fig. 3E–H), viz. "*Hydrobioides nassa rivicola*" (Fig. 3O), mostly found in rivers. However, there is no clear boundary between these two morphotypes. The syntype is quite similar to the "*lacustris* "type but much bigger than the newly collected specimens (Fig. 3A–D). This species was considered widely distributed in north to central Thailand. However, "*Hydrobioides nassa*" from Thailand does not have an obvious varix and an extended outward outer lip, and the molecular analysis has confirmed that the Thailand species is distinct from *Hydrobioides nassa* from the type locality, Shan State of Myanmar (Fig. 2). Therefore, we consider that *Hydrobioides dautzenbergi* Walker, 1927, stat. rev. (type locality: Thailand, Chieng Mai) should be a valid species name for the "*Hydrobioides nassa*" from Thailand. *Hydrobioides nassa* is probably endemic to Shan State, Myanmar.

Habitat and distribution. Rivers, ponds, wetlands, and lakes of Shan State, Myanmar.

Values are arithmetic means, standard deviations, maximum and minimum values (in brackets) of shell height (H), shell width (W), and aperture height (AH) of *n* measured specimens.

Hydrobioides physcus Annandale, 1918, comb. rev.

Hydrobioides physcus Annandale, 1918: 121–122, pl. 13, figs 8, 8a, 9, pl. 14, figs 5, 5a (Myanmar, Shan State, Inle Lake).
Hydrobioides (*Paranerita*) *physcus*–Annandale, 1920: 45.
Paranerita physcus – Annandale & Rao, 1925: 115.
Parabithynia physcus – Pilsbry, 1928: 108
Gabbia physcus – Subba Rao, 1989: 78, figs 127–129.

Diagnosis. Shell medium, low spiral whorls, weak keel on shoulder, outer lip outward extended.

Material examined. • 3 probable ***paratypes*** (preserved dry), Inle Lake, South Shan, Burma. "Ex. India Museum", NHMUK.20060144; 2 specimens (preserved in ethanol), Shan State, Taunggyi District, Inn Paw Hkon, Inthein, riverbank under Inthein Bridge (Fig. 1B. d), 20°27'35.9"N, 96°50'32.6"E, 1 Jul. 2024, Le-Jia Zhang leg., KIZ.2400061–2400062 • 2 specimens (preserved in ethanol), Shan State, Taunggyi District, Nyaungshwe, Inle Lake, floating islands near Intha (Fig. 1B. c), 20°36'33.4"N, 96°54'53.8"E, 3 Jul. 2024, Le-Jia Zhang leg., KIZ.2400063–2400064.

Description. Shell (Fig. 7) medium for the family (Table 1), relatively thick, solid, subglobose to globose, white or bright yellow to orange yellow, with four whorls at adulthood; teleoconch low, with a keel on shoulder, forming weak nodules on shoulder with vertical growth lines, two weak additional keels on body whorl; aperture ovate, lip thickened, always more than half of shell in height, outer lip outward extended; umbilicus covered by inner lip completely.

Operculum (Figs 7A, 4B) calcium, ovate, thin, slightly smaller than aperture, transparent grey; exterior surface smooth, nucleus close to the central of operculum; interior surface with relatively thin outer opercular region, narrow weak concentric veins on the margin of inner opercular region, nuclear region relatively large, with irregular veins and grains.

Penis (Fig. 5B) with a white penial appendix located in the central of penis, penial appendix slightly shorter than distal part of penis.

Radula (Fig. 8) taenioglossate; central tooth upper margin with one broad triangle central cusp and two to three small sharp cusps on either side, lateral margin each with two to three small sharp cusps; lateral tooth with one broad tongue-shape central cusp and two to three small sharp cusps; inner marginal tooth with 10 to 11 small cusps; outer marginal tooth with six small cusps.

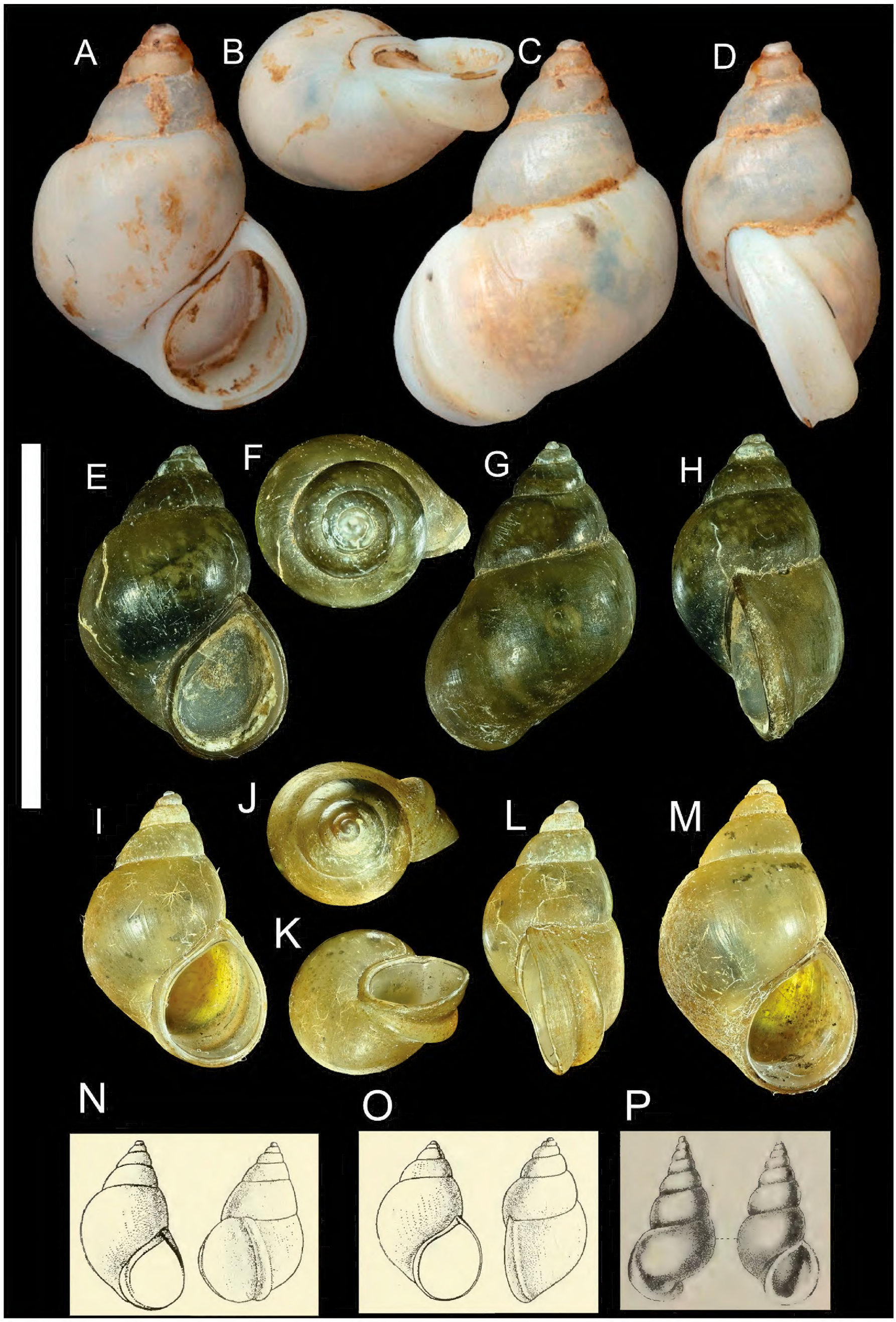

Figure 3. Shell of *Hydrobioides nassa* (**A–O**) and *Hydrobioides turrita* (P). **A–D**. NHMUK.1888.12.4.845, Syntype of *Bithinia nassa*, from Natural History Museum (2014); **E–H.** KIZ.2400057, Kalaw; I–M, KIZ.2400054 (I–L), 2400055 (M), Inle Lake; **N.** "*Hydrobioides nassa lacustris*" from Annandale, 1918; **O.** "*Hydrobioides nassa rivicola*" from Annandale, 1918; **P.** "Bythinea(?) trrita" from Nevill (1881). Scale bars: 1 cm (**A–I**).

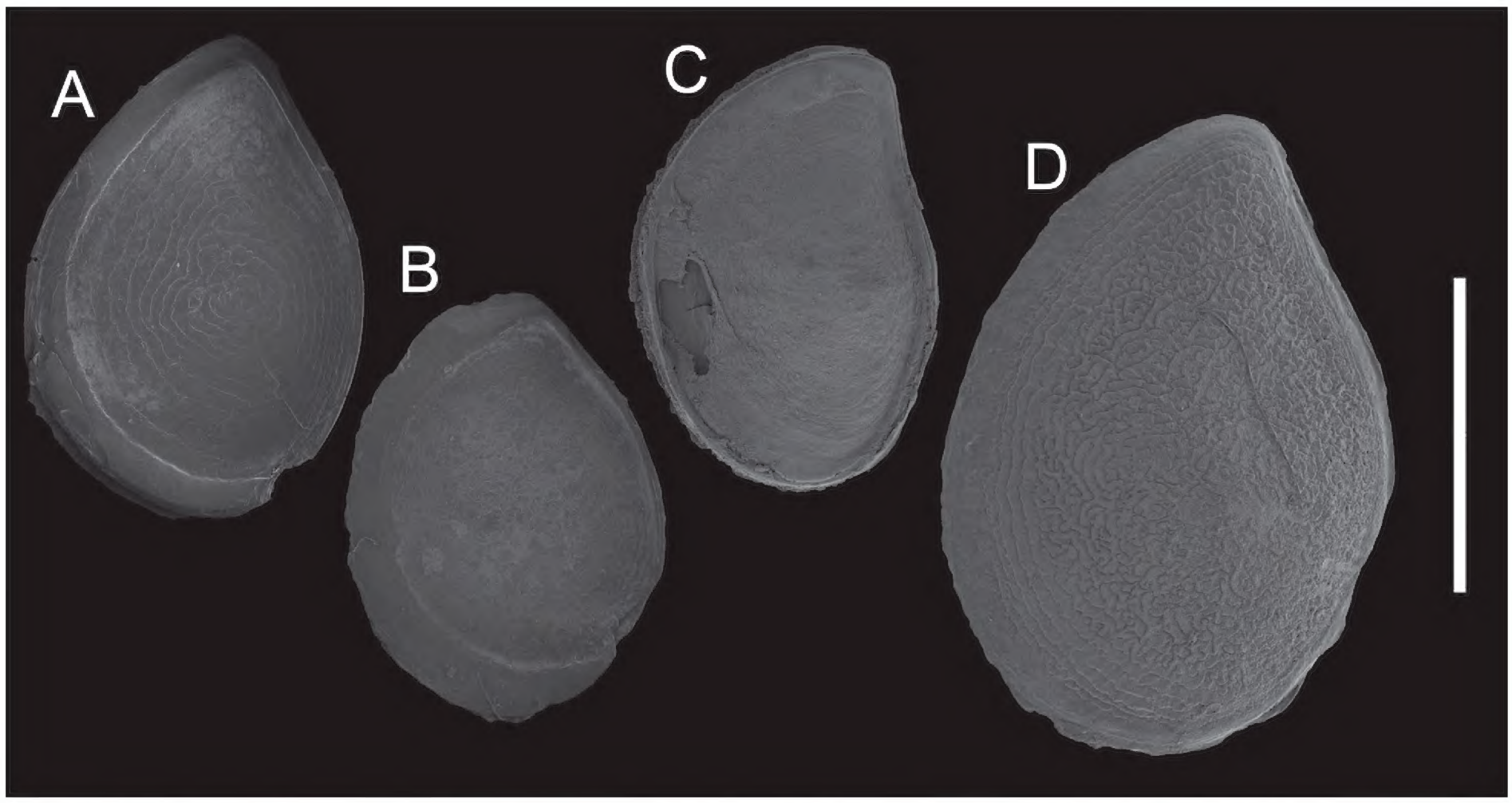

Figure 4. SEM photo of the opercular inner surface of bithyniid snails from the Inle Lake basin. **A.** *Hydrobioides nassa*; **B.** *Hydrobioides physcus*; **C.** *Gabbia nana*; **D.** *Digoniostoma iravadica*. Scale bars: 1 mm.

Table 1. Shell measurements of bithyniid snails from the Inle Lake basin.

Species	*n*	H	W	AH	W/H	AH/H
Hydrobioides nassa	6	7.97 ± 0.76 (6.57–8.87)	5.23 ± 0.49 (4.3–5.69)	4.23 ± 0.35 (3.67–4.57)	0.66 ± 0.01 (0.64–0.69)	0.53 ± 0.03 (0.5–0.57)
Hydrobioides physcus	4	5.94 ± 0.27 (5.52–6.18)	5.19 ± 0.11 (5.08–5.36)	4.02 ± 0.20 (3.75–4.29)	0.88 ± 0.03 (0.84–0.92)	0.68 ± 0.03 (0.64–0.7)
Hydrobioides avarix	2	(6.57–6.7)	(4.43–4.48)	(3.42–3.48)	(0.67)	(0.52)
Gabbia nana	9	4.54 ± 0.38 (3.74–5.15)	3.44 ± 0.27 (2.87–3.77)	2.64 ± 0.20 (2.23–2.92)	0.76 ± 0.02 (0.73–0.79)	0.58 ± 0.02 (0.57–0.62)
Digoniostoma iravadica	6	12.06 ± 0.42 (11.42–12.65)	6.91 ± 0.22 (6.55–7.28)	6.22 ± 0.29 (5.82–6.65)	0.57 ± 0.02 (0.55–0.61)	0.52 ± 0.02 (0.48–0.53)

Remarks. This species can be easily distinguished from other bithyniid snails based on the thick subglobose to globose shell with keels and shoulder. It also can be distinguished from *H. nassa* based on opercular characters and less small cusps on inner and outer marginal teeth. This species is the type species and only extant species of genus *Parabithynia* Pilsbry, 1928, which is now endemic to Inle Lake. Several fossil species of this genus were reported from China and Russia (Popova 1981; Yu et al. 1978), but the assignment needs a revision. Our molecular study confirms that this species is the sister species of *H. nassa*, with a close genetic relationship (p distance of 16S: 4.3–4.5%). The similarity in shell (especially the extended outer lip), operculum, and male genital structure also supports that these two species should be placed in one genus. Therefore, we consider that *Parabithynia* is a synonym of *Hydrobioides*. *Hydrobioides physcus* comb. rev. should be re-assigned to its original genus.

Habitat and distribution. Inle Lake and the big rivers connected to Inle Lake; swamps in Heho, Shan State, Myanmar.

Hydrobioides avarix Annandale, 1918

Hydrobioides avarix Annandale, 1918: pl. 14, figs 1, 2, 2a, 2b, 2c (Myanmar, Shan State, NW shore of Inle Lake, a stream near Fort Stedman, now Maing Thauk).

Material examined. • 3 probable ***syntypes*** (preserved dry), Inle Lake, South Shan, Burma. "Ex. India Museum", NHMUK.20060143.

Remarks. We did not discover this species during our fieldwork and only examined three probable syntypes. The detailed descriptions of shell, operculum, radula, and male genitalia of this species have been given by Annandale (1918). This species can be distinguished from *Hydrobioides nassa* by more inflated whorls and a simple outer lip without the varix structure nearby. It can be distinguished from *Gabbia nana* comb. nov. based on a larger and thinner shell with less inflated whorls and an operculum, which is much smaller than the aperture. The validity and genus assignment of this species need further study based on fresh specimens.

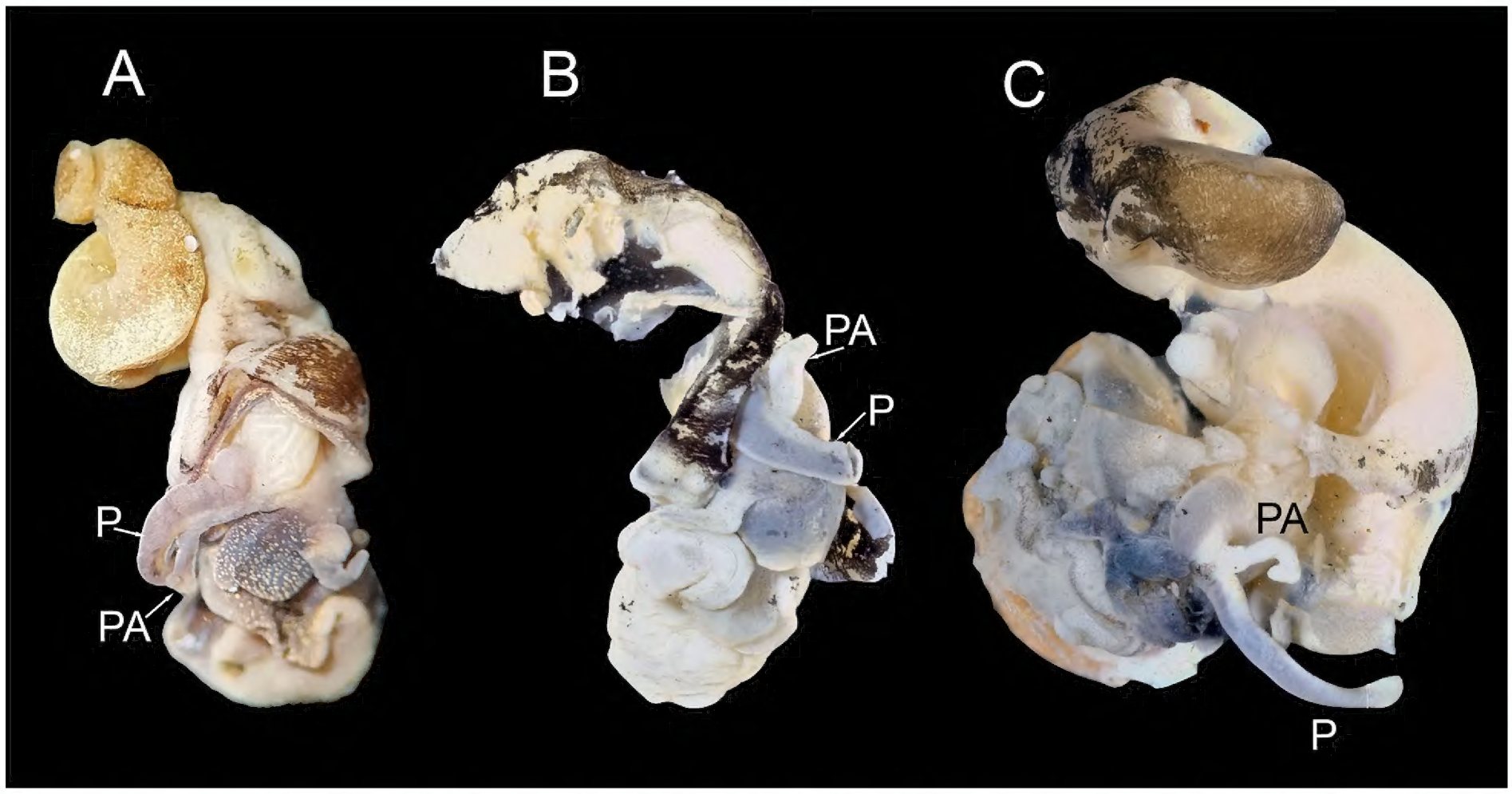

Figure 5. Male genitalia of bithyniid snails from the Inle Lake basin. **A.** *Hydrobioides nassa*; **B.** *Hydrobioides physcus*; **C.** *Digoniostoma iravadica*. P refers to penis; PA refers to penial appendix.

Gabbia Tryon, 1865

Type species. *Gabbia australis* Tryon, 1865 accepted as *Gabbia vertiginosa* (Frauenfeld, 1862) (type by monotypy; Australia, New South Wales).

Remarks. This genus is a highly diverse genus with nearly 50 species. Most species were assigned to this genus only based on the morphology of a small globose shell with an inflated body whorl. The taxonomy of this genus needs a systematic revision using molecular methods, especially a large number of species from Australia (including the type species *Gabbia vertiginosa*).

Gabbia nana (Annandale 1918), comb. nov.

Hydrobioides nana Annandale, 1918: pl. 14, fig. 3 (Myanmar, Shan State, NW shore of Inle Lake, Fort Stedman, now Maing Thauk).

Diagnosis. Shell small, transparent white to pale yellow, high spiral whorls, inflated body whorl.

Material examined. • 10 specimens (preserved in ethanol), Shan State, Taunggyi District, Nampan, water in rice field (Fig. 1B. e), 20°28'02.6"N, 96°55'58.1"E, 5 Jul. 2024, Le-Jia Zhang leg., KIZ.2400065–2400075.

Description. Shell (Fig. 9) small for the family (Table 1), thin but solid, subglobose, transparent white to pale yellow, with four to 4.5 whorls at adulthood; teleoconch relatively high, smooth, without shoulder, body whorl inflated; aperture ovate, lip slightly thickened, always more than half of shell in height; umbilicus covered by inner lip or with narrow slit.

Operculum (Figs 9A, F, 4C) calcium, ovate, thin, slightly bigger than aperture, transparent grey; exterior surface smooth, nucleus close to inner opercular margin; interior surface with weak concentric growth lines and small grains on inner opercular region.

No fresh male specimen discovered.

Radula (Fig. 10) taenioglossate; central tooth upper margin with one long triangle central cusp and three small sharp cusps on either side, lateral margin each with three small sharp cusps; lateral tooth with one long triangle central cusp and four small sharp cusps; inner marginal tooth with 16 to 17 small cusps; outer marginal tooth with nine small cusps.

Remarks. This species is assigned to *Gabbia* based on its morphology of a small globose shell with an inflated body whorl and its close relationship with *Gabbia pygmaea* in the molecular phylogeny (Fig. 2). It cannot be assigned to *Hydrobioides* since this species lacks an outward extended outer lip. It can be distinguished from *Gabbia pygmaea* (Preston, 1908) (type locality: Myanmar, Thayet) based on its higher spiral whorls and white to pale yellow shell.

Habitat and distribution. Only known from the wetlands and small creeks in the Inle Lake basin.

Digoniostoma Annandale, 1920

Digoniostoma Annandale, 1920 in Annandale & Seymour Sewell (1920): 103–104; Starobogatov, 1970: 26; Izzatullaev, 1982: 336–340; Rao, 1989: 79–80; Ng & Tan, 2024: 154–155.

Bithynia (*Digoniostoma*) – Brandt, 1974: 58; Ramakrishna, 2007: 118–122; Glöer & Bössneck, 2013: 137–156.

Bithynia – Molluscabase, 2024b: synonym.

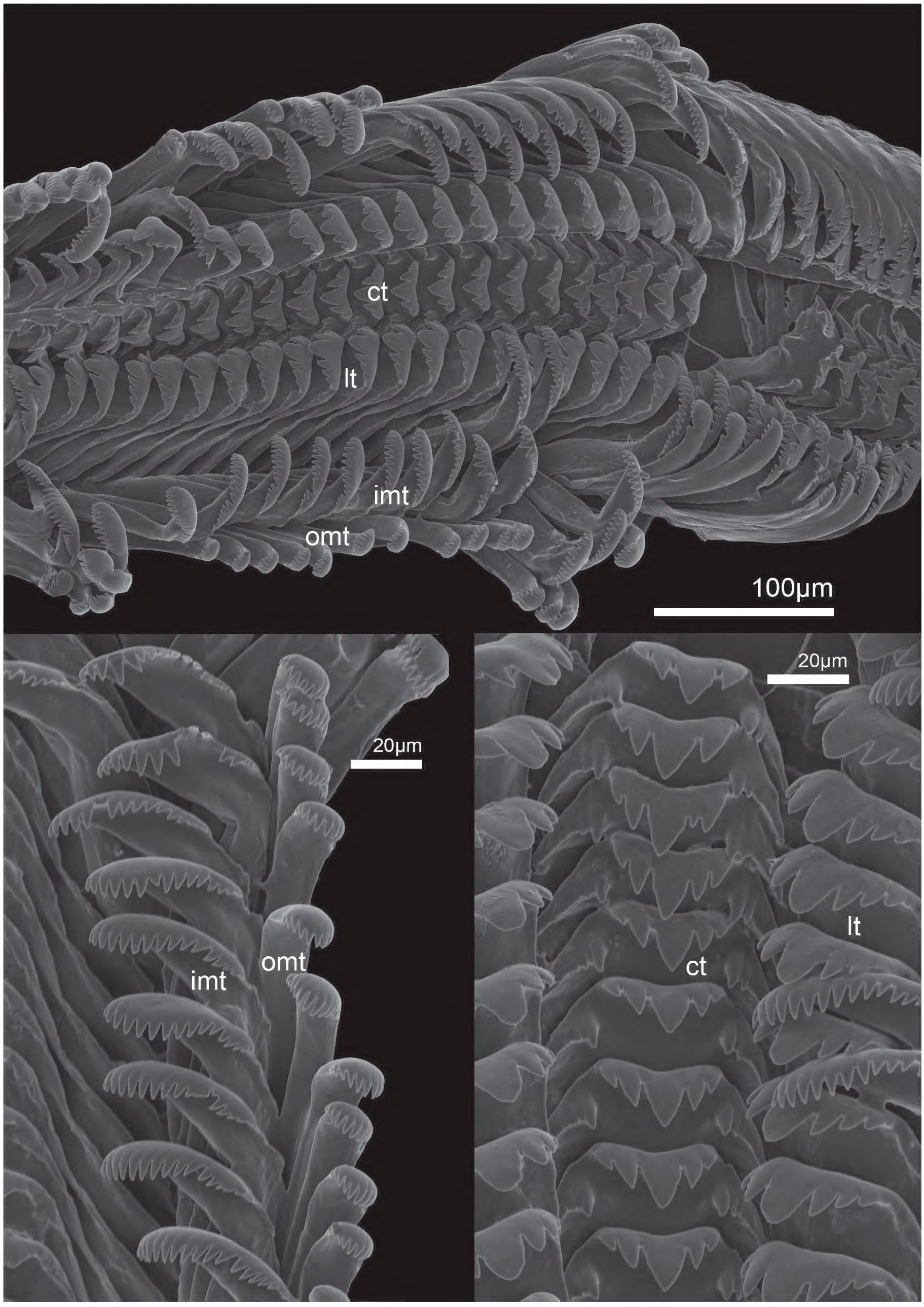

Figure 6. SEM photo of the radula of *Hydrobioides nassa*.

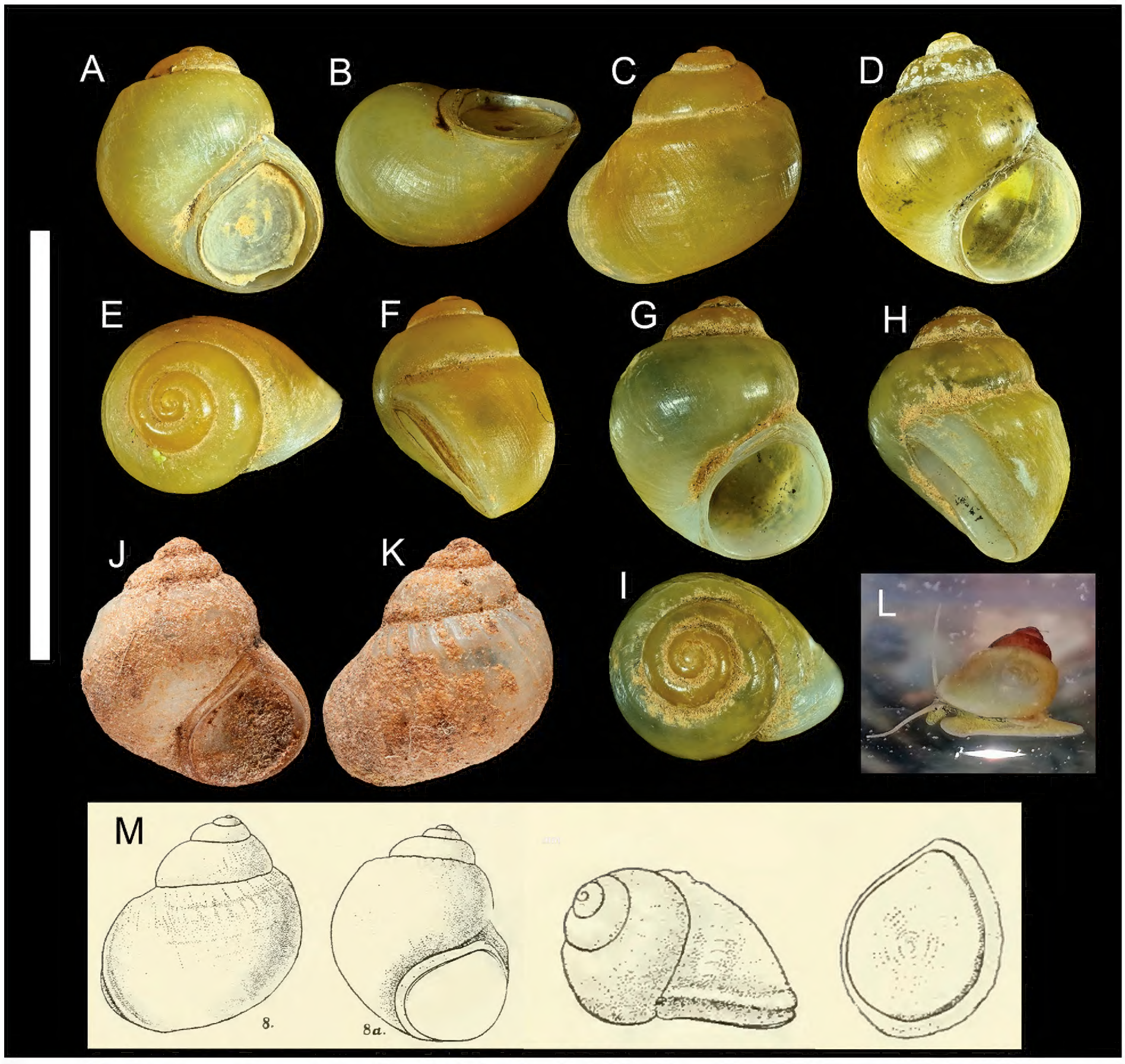

Figure 7. Shell of *Hydrobioides physcus*. **A–C.** KIZ.2400063, Inle Lake; **D.** KIZ.2400064, Inle Lake; **E–F.** KIZ.2400063, Inle Lake; **G–I.** KIZ.2400063, Inthein; **J–K.** Probable paratype, NHMUK.20060144, taken by Aimee McArdle, NHMUK Photographic Unit; **L.** Living animal in aquarium; **M.** Drawings of the type specimens from Annandale, 1918. Scale bars: 1 cm (**A–I**).

Type species. *Paludina cerameopoma* Benson, 1830 (original designation).

Diagnosis. Shell large for the family, thin and fragile, inner lip straight, base of peristome angled, umbilicus open, usually with a keel around; penial appendix located close to the base of penis, penial appendix much shorter than distal part of penis.

Remarks. This genus is always considered as a subgenus or even a synonym of *Bithynia* by some recent studies, e.g., Glöer and Bössneck (2013) and Molluscabase (2024b). However, the molecular study has confirmed that *Digoniostoma* from South and Southeast Asia is a distinct group and not closely related to *Bithynia*, mainly from Europe. Morphologically, this genus also can be easily distinguished from other bithyniid groups based on large shell with base of peristome angled and open umbilicus with a keel around. Molecular, morphological, and distributional evidence all support that *Digoniostoma* is a valid genus. There are 11 species and subspecies assigned to this genus based on molecular evidence and former morphological studies: *Digoniostoma cerameopoma* (Benson, 1830); *Digoniostoma funiculata* (Walker, 1927), stat. nov.; *Digoniostoma kashmirense* (G. Nevill, 1885); *Digoniostoma lithoglyphoides* Nesemann & Sharma, 2007; *Digoniostoma oxiana* Izzatullaev, 1982; *Digoniostoma pulchella* (Benson, 1836); *Digoniostoma iravadica* (Blanford, 1869); *Digoniostoma siamensis siamensis* (Lea, 1856), stat. nov.; *Digoniostoma siamensis goniomphalos* (Morelet, 1866) stat. nov.; *Digoniostoma textum* Annandale, 1921; *Digoniostoma truncatum* (Eydoux & Souleyet, 1852). The taxonomy of *Digoniostoma* species, especially the species from India, should be revised.

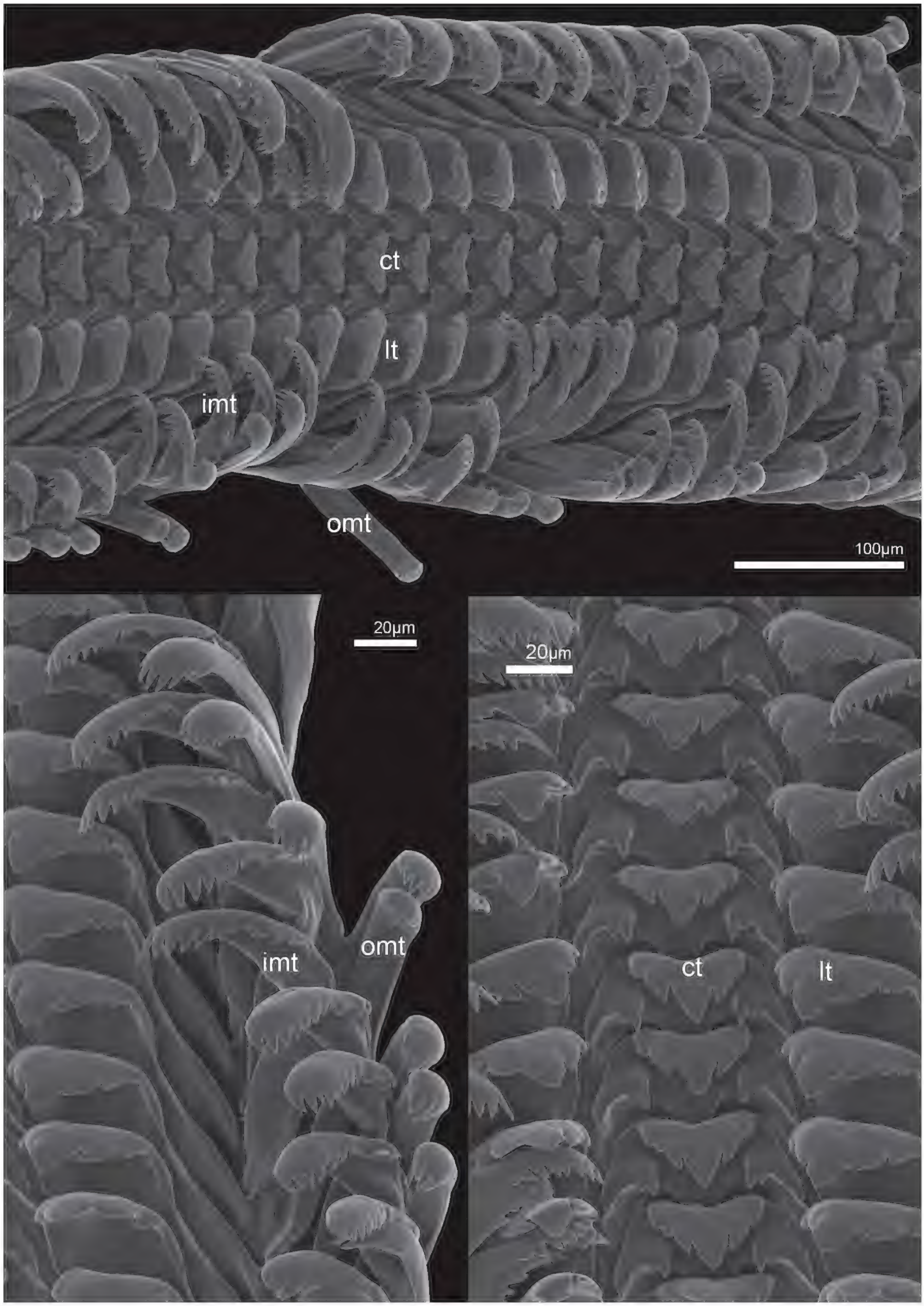

Figure 8. SEM photo of the radula of *Hydrobioides physcus*.

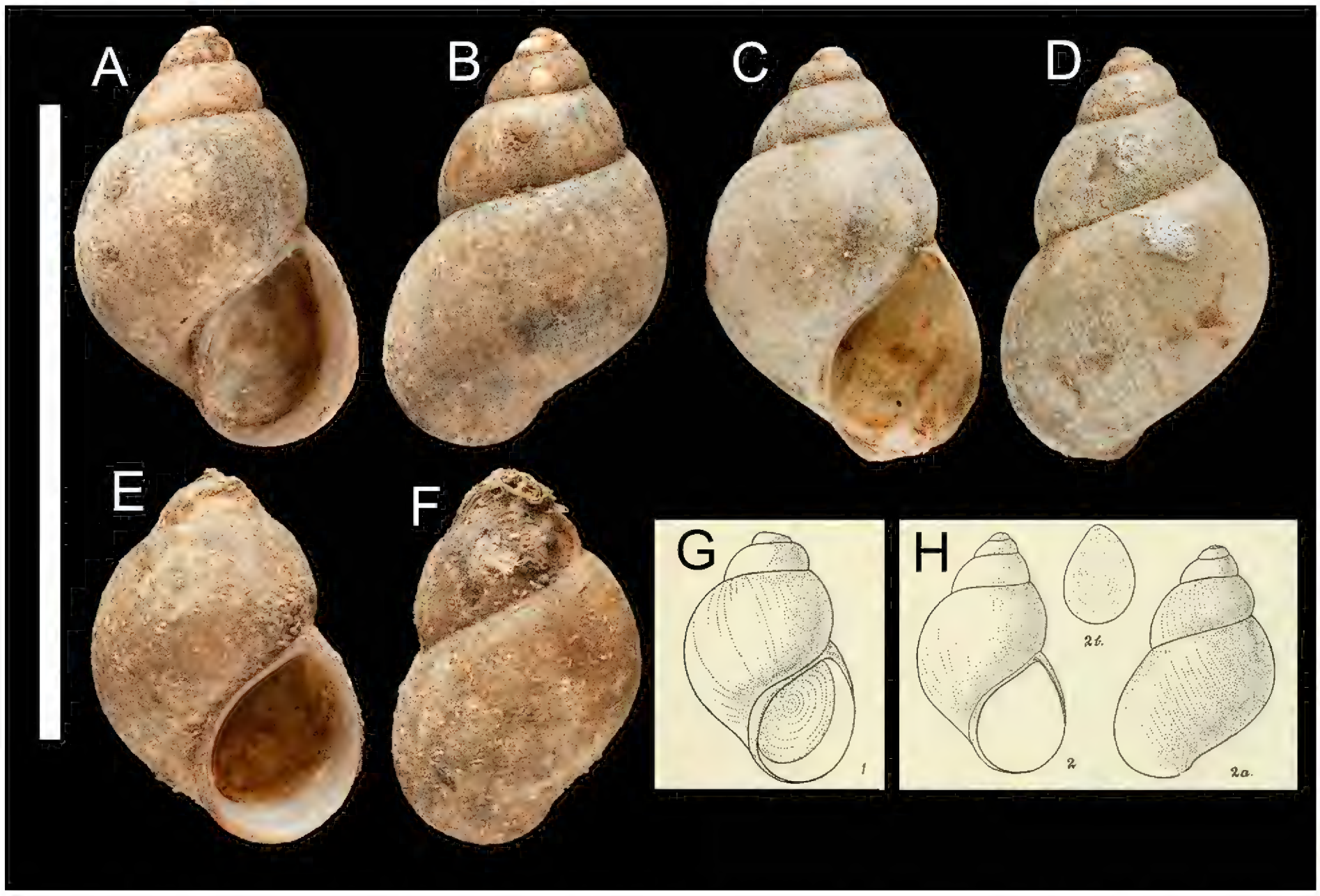

Figure 9. Shell of *Hydrobioides avarix*. **A–F.** Probable syntypes, NHMUK 20060143, taken by Aimee McArdle, NHMUK Photographic Unit; **G–H.** Drawing of the type specimens of *Hydrobioides avarix* from Annandale, 1918. Scale bars: 1 cm (**A–F**).

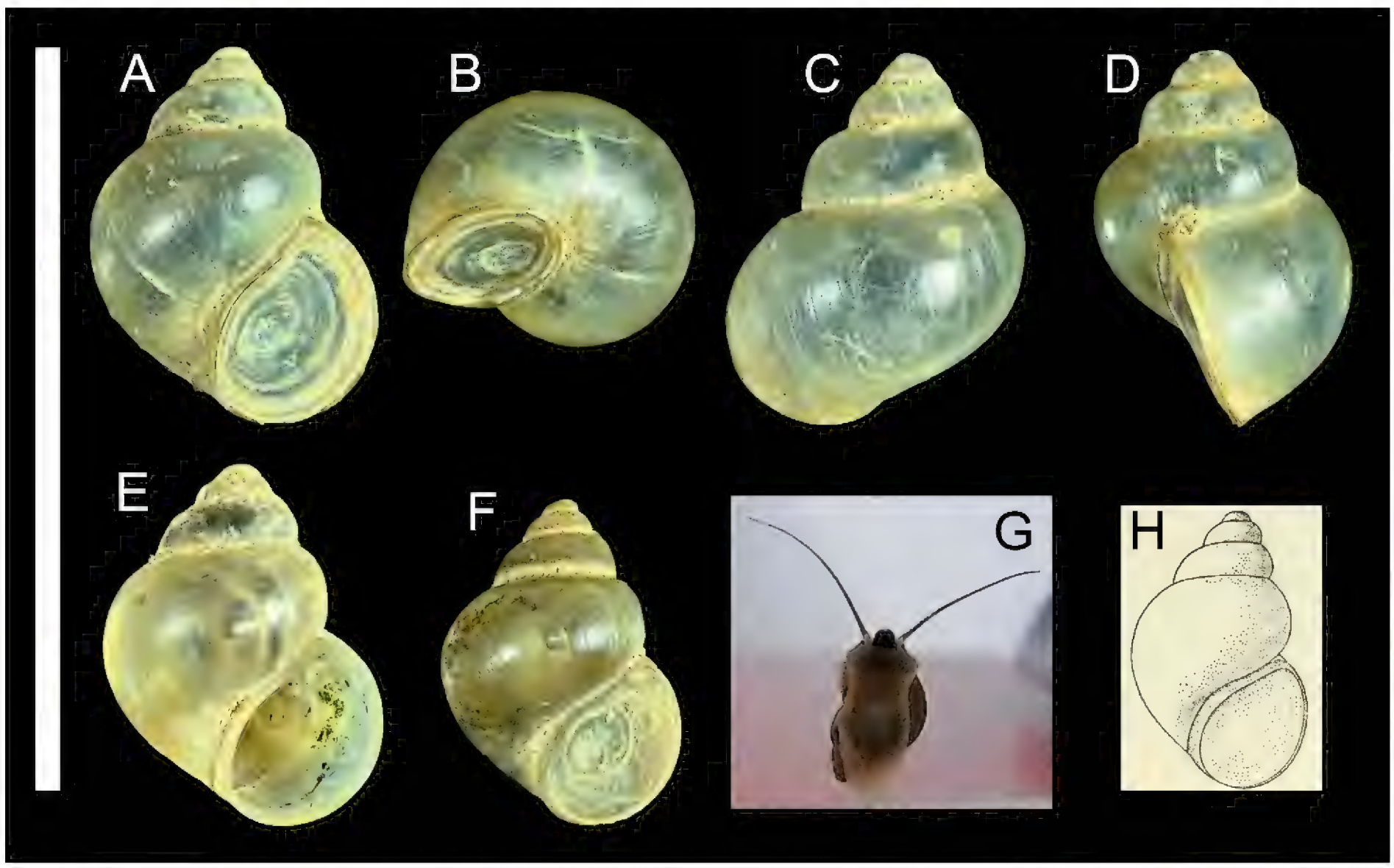

Figure 10. Shell of *Gabbia nana*. **A–D.** KIZ. 2400065, Nampan; **E.** KIZ. 2400066, Nampan; **F.** KIZ. 2400067, Nampan; **G.** Living animal in aquarium; **H.** Drawing of the type specimens of *Hydrobioides nana* from Annandale, 1918. Scale bars: 1 cm (**A–F**).

Digoniostoma iravadica (Blanford, 1869), comb. nov.

Bithynia iravadica Blanford, 1869: 446 (Myanmar, Mandalay, Ava, swamp, and river); Molluscabase, 2021: taxon inquirendum.

D. iravadica – Rao, 1989: no description or remarks, only figures with ambiguous genus assignment and species name, figs 122–123.

Diagnosis. Shell big, thin, high spiral whorls, thin outer lip, base of peristome angled, umbilicus narrow and deep, penial appendix about one-third of distal part of penis in length.

Material examined. • 7 specimens preserved in ethanol, Shan State, Taunggyi District, Shwenyaung, Taung Lay Lone, a shallow pond in the north of town (Fig. 1B. a), 20°44'56.7"N, 96°53'59.4"E, 6 Jul. 2024, Le-Jia Zhang leg., KIZ.2400076–2400082 • 1 dry shell specimen, Mandalay Region, Meiktila, Wundwin, Seywa, dry pond near road, 21°14'15.2"N, 95°50'27.7"E, 29 Jun. 2024, Le-Jia Zhang leg., KIZ.2400083.

Description. Shell (Fig. 11) relatively large for the family (Table 1), thin and fragile, high conical, pale olive green to grey, with 5.5 whorls at adulthood; teleoconch high, relatively smooth but with very fine grid-like micro-structure, without shoulder; aperture ovate, nearly half of shell in height, outer lip thin, inner lip straight, base of peristome angled; umbilicus narrow and deep, always open obviously, with a keel around.

Operculum (Figs 11A, G, 4A) calcium, ovate, thin, slightly smaller than aperture, transparent grey; exterior surface smooth, with obviously concentric growth lines, nucleus close to the inner opercular margin; interior surface with many vermicular veins covering most parts of inner opercular region, and a few concentric veins on the margin of inner opercular region.

Penis (Fig. 5C) with a white penial appendix located close to the base of penis, penial appendix about one-third of distal part of penis in length.

Radula (Fig. 12) taenioglossate; central tooth upper margin with one long triangle central cusp and four small sharp cusps on either side, lateral margin each with three small sharp cusps; lateral tooth with one long triangle central cusp and four small sharp cusps; inner marginal tooth with 10 to 11 small cusps; outer marginal tooth with 10 small cusps.

Remarks. This species can be distinguished from other *Digoniostoma* species based on high spiral whorls, fine grid-like micro-structure shell surface, narrow umbilicus, and very long distal part of the penis. The molecular study also confirms the validity of this species. The radula of *Digoniostoma iravadica* has the central cusp with a different shape and a larger number of small sharp cusps on both the central tooth and the lateral tooth, compared to that of *Hydrobioides* species from Inle Lake.

Habitat and distribution. Rivers, swamps, and shallow ponds in central Myanmar (Mandalay, Meiktila, Taunggyi).

Discussion

Ancient lakes are widely recognised as important refuges and evolutionary centres for aquatic fauna. As the only ancient lake on the Indochinese Peninsula, Inle Lake supports a high diversity of bithyniid snails. The five bithyniid species examined in the present study are all endemic to Myanmar, with three species (*Hydrobioides physcus*, *Hydrobioides avarix*, and *Gabbia nana*) restricted to the Inle Lake basin and nearby region (Heho). We did not find two tiny bithyniid species endemic to the Inle Lake basin, viz. *Hydrobioides avarix* and *Gabbia alticola* (Annandale 1918). Based on the original description and the type specimens figured by Sawada (2022), we considered *Gabbia alticola* a valid endemic species. With altogether four endemic bithyniid species, the Inle Lake basin appears to have the highest known endemicity of Bithyniidae worldwide, surpassing Lake Skadar and Lake Trichonis of the Balkan Peninsula, each with three endemic bithyniid species (Glöer et al. 2007). In total, including the newly recorded *Digoniostoma iravadica*, seven bithyniid species have been recorded from the Inle Lake basin. Our study confirms the continued existence of four species and provides the first molecular-based revision of the bithyniid fauna from the Inle Lake basin.

Our multi-method study has significantly improved the taxonomy of bithyniid snails. *Hydrobioides nassa* has long been considered a common species in northern to central Thailand (Brandt 1974; Kulsantiwong et al. 2013; Bunchom et al. 2021). However, all previous studies did not compare the Thai population to the population from the type locality, Shan State of Myanmar. Our present study confirms that the true *Hydrobioides nassa* from Shan State can be clearly distinguished from the Thai "*Hydrobioides nassa*" based on shell morphology and molecular phylogeny. The molecular tree has revealed that the Thai "*Hydrobioides nassa*" is not even closely related to *Hydrobioides nassa*, and the sister species of *Hydrobioides nassa* is *Hydrobioides physcus*, endemic to Inle Lake. Therefore, we reintroduce the name *Hydrobioides dautzenbergi* for the Thai species, though its genus assignment requires further study due to the polyphyletic condition of *Hydrobioides* (Fig. 2). Genus *Parabithynia* with the type species *Hydrobioides physcus* is considered a synonym of *Hydrobioides* based on strong evidence of molecular study and morphology. *Digoniostoma* should be a valid genus based on molecular, morphological, and distributional evidence.

The present study on Bithyniidae represents the first part of our systematic survey and study of freshwater snails from the Inle Lake basin. As an important biodiversity hotspot of freshwater fauna, Inle Lake is increasingly threatened by human activities (Iwai et al. 2022). Further research on other freshwater snails from the Inle Lake region will enhance our understanding of the biodiversity and evolution of freshwater snails in this ancient plateau lake and provide valuable insights for the conservation of Inle Lake.

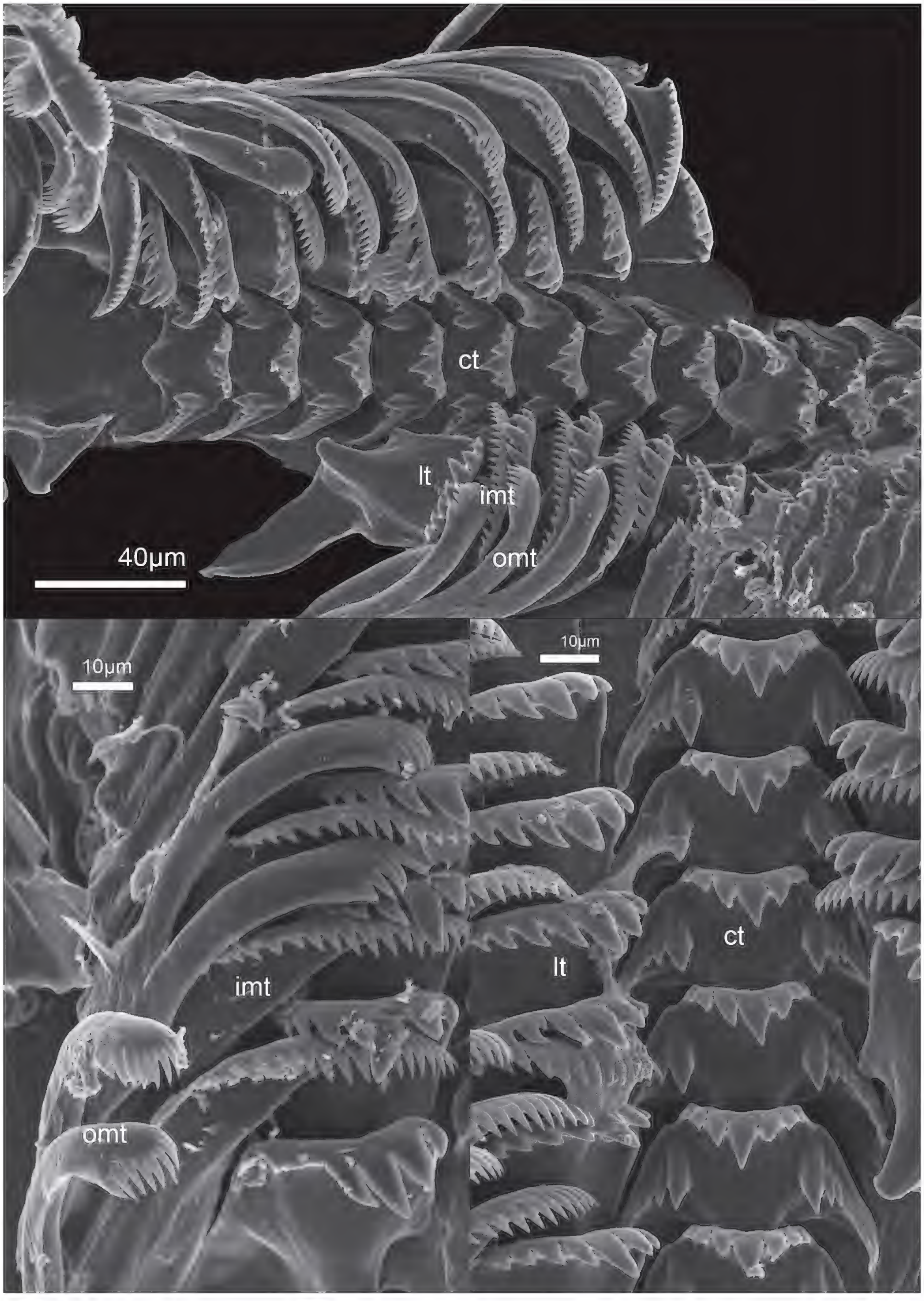

Figure 11. SEM photo of the radula of *Gabbia nana*.

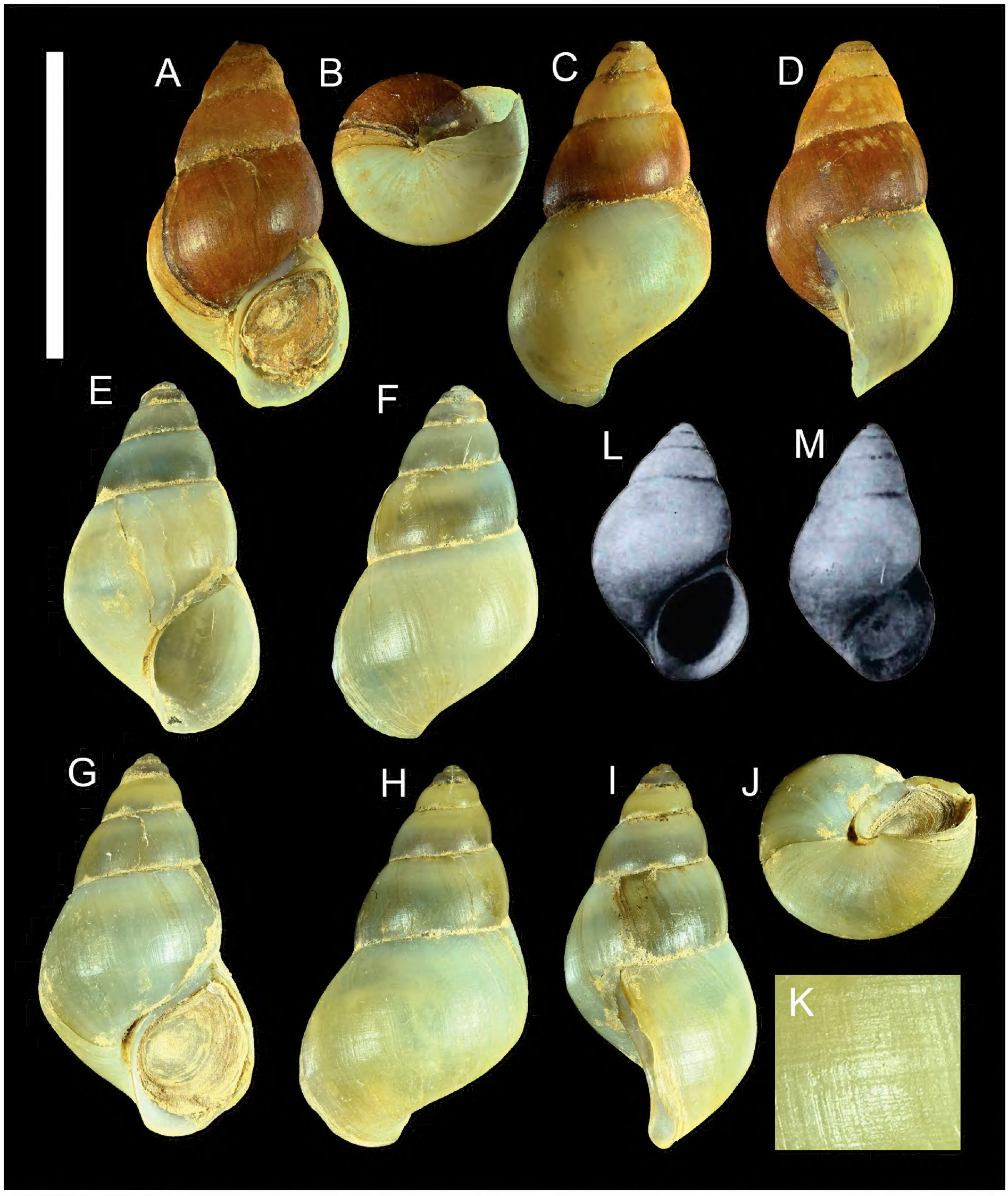

Figure 12. Shell of *Digoniostoma iravadica*. **A–D.** KIZ.2400076, Shwenyaung, dyed brown by the minerals; **E–F.** KIZ.2400077, Shwenyaung; **G–J.** KIZ.2400078, Shwenyaung; **K.** Micro-structure on shell surface; **L–M.** Figure from Rao (1989). Scale bars: 1 cm (**A–J**).

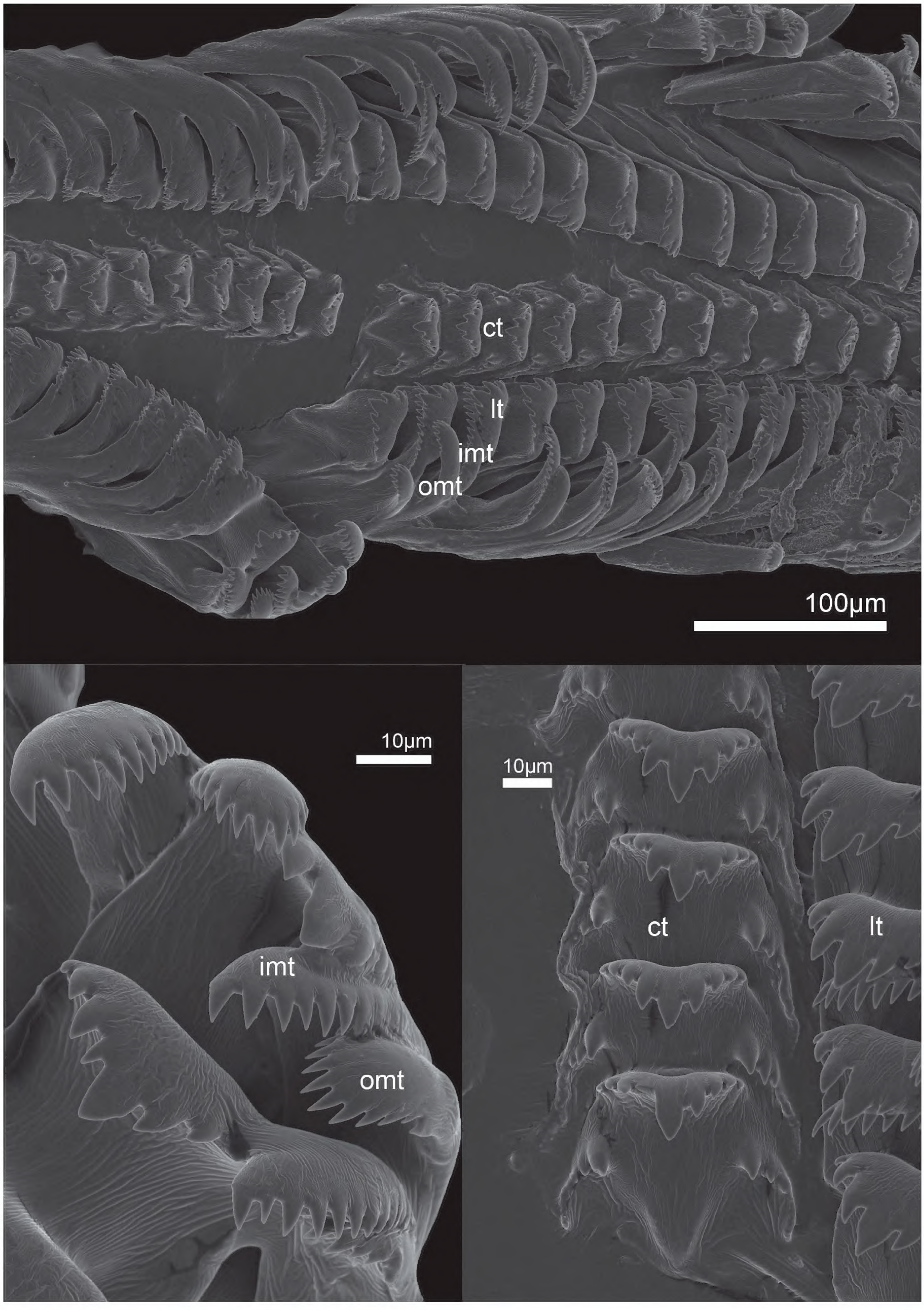

Figure 13. SEM photo of the radula of *Digoniostoma iravadica*.

Acknowledgements

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Supplementary material 1

Supplementary data

Authors: Le-Jia Zhang, Shu-Sen Shu, Xin-Yuan Song, Nay Htet Naing, Thaung Naing Oo, Xiao-Yong Chen

Data type: xlsx

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